DEVELOPMENT AND VALIDATION OF A SENSITIVE SPE/LC/MS METHOD FOR THE DETERMINATION OF GLUCOSAMINE IN DOG PLASMA

Hubert, C., Liège/B, Houari, S., Liège/B, Houbart, V., Liège/B, De Bleye, C., Liège/B, Fillet, M., Liège/B, Rozet, E., Liège/B, Hubert, Ph., Liège/B

PhD student Cedric Hubert, Laboratory of Analytical Chemistry, CIRM, Institute of Pharmacy, University of Liège, B 36, B-4000 Liège, Belgium

The present LC/MS method was developed for the monitoring of dog plasmatic concentration of glucosamine (GLcN). In this scope, relatively low plasmatic concentrations of GLcN were expected, ranging from 50 ng/mL to 1000 ng/mL. As this method must be the most sensible, liquid chromatography coupled to simple quadrupole mass spectrometry detection (LC/MS) was selected thanks to its efficiency and usefulness for this objective. Additionally, a solid phase extraction (SPE) step was performed to avoid matrix effect as well as ion suppression.

Due to the ionisable character of the compound of interest, a mixed-mode strong cation exchange (Plexa PCX) disposable extraction cartridge (DEC) was selected. 350 µL of the plasma sample was first acidified with 35 µL of trichloroacetic acid (10%). 25 µL of miconazole, the internal standard (IS), at 1000 ng/mL were then added. 325 µL of resulting sample were treated. The cartridges were successively conditioned with 1 mL of acetonitrile and 500 µL of a 20 mM formic buffer at pH=3.0. The washing step was realized with 500 µL of acetonitrile. The elution was led using 1 mL of a mixture of acetonitrile and 5% ammonia (70/30, v/v). Following this extraction, the solution was evaporated to dryness and the residue was recovered in 150 µL of a 5 mM ammonium hydrogen carbonate buffer at pH=7.5. Finally, 20 µL of this last solution were injected into the chromatographic system.

The separation was carried out on a Agilent Zorbax SB-CN column (250 x 4.6, 5 µm) using a mobile phase consisting in a mixture of methanol and 5 mM ammonium hydrogen carbonate buffer at pH=7.5 (95/5, v/v). The detection was led at a m/z ratio 180.0 and 417.0 for GLcN and IS respectively. Reliability of the results was demonstrated through the validation of the method using an approach based on the accuracy profile [1-2] allowing to manage the risk associated to the use of these methods in routine analysis [3]: it is thus guaranteed that each future results will fall in the +/-30% acceptance limits with a probability of at least 95%.

This study was sponsored by KitoZyme sa (Belgium).

[1] PAT Initiative, FDA, http://www.fda.gov/cder/OPS/PAT.htm

[2] Ph. Hubert et al, Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal—part I, J. Pharm. Biomed. Anal. Vol. 36, 579 (2004).

[3] ICH guidelines Q9, EMEA, http://www.emea.eu.int/Inspections/docs/ICHQ9Step4QRM.pdf